Applicant: GSF-Forschungszentrum für Umwelt und Gesundheit GmbH
Neuherberg
Ingolstädter Landstraße 1
85764 Oberschleißheim

Method for ex vivo immunization using heterologous intact bispecific and/or trispecific antibodies

C L A I M S

1. Method for ex vivo immunization of humans and animals comprising the following steps of:

a) isolating autologous tumour cells;

b) treating the tumour cells to prevent the survival thereof following reinfusion;

c) incubating the thus treated tumour cells with intact heterologous bispecific and/or trispecific antibodies showing the following properties:

α - binding to a T cell;

ß - binding to at least one antigen on a tumour cell;

γ - binding, by their Fc portion (in the case of bispecific antibodies), or by a third specificity (in the case of trispecific antibodies) to Fc receptor-positive cells.

Sub B1

2. Method according to claim 1,
characterized in that
said antibodies are selected so that they are capable of binding Fc receptor-positive cells having a Fcγ receptor I, II, or III.

3. Method according to claim 2,
characterized in that
said antibodies are capable of binding to monocytes, macrophages, dendritic cells, "natural killer" cells (NK cells) and/or activated neutrophils being Fcγ receptor I-positive cells.

4. Method according to claim 1,
characterized in that
said antibodies are capable of inducing tumour-reactive complement-binding antibodies and thus inducing a humoral immune response.

5. Method according to claim 1,
characterized in that
said antibodies are selected to bind to the T cells via CD2, CD3, CD4, CD5, CD6, CD8, CD28 and/or CD44.

6. Method according to claim 1,
characterized in that
said antibodies are selected so that following their

binding to the Fc receptor-positive cells the expression of CD40, CD80, CD86, ICAM-1 and/or LFA-3 as co-stimulatory antigens, and/or secretion of cytokins by the Fc receptor-positive cell is initiated or increased.

7. Method according to claim 6,
characterized in that
said antibodies are selected so that the secretion of IL-1, IL-2, IL-4, IL-6, IL-8, IL-12 being cytokins and/or of TNF-α is increased.

8. Method according to claim 1,
characterized in that
said bispecific antibody is selected to be an anti-CD3 X anti-tumour-associated antigen antibody and/or anti-CD4 X anti-tumour-associated antigen antibody and/or anti-CD5 X anti-tumour-associated antigen antibody and/or anti-CD6 X anti-tumour-associated antigen antibody and/or anti-CD8 X anti-tumour-associated antigen antibody and/or anti-CD2 X anti-tumour-associated antigen antibody and/or anti-CD28 X anti-tumour-associated antigen antibody and/or anti-CD44 X anti-tumour-associated antigen antibody.

9. Method according to claim 1 ~~one or more of the preceding claims~~,
characterized in that
said bispecific antibody is selected from one or more of

the following isotype combinations:

rat-IgG2b/mouse-IgG2a,

rat-IgG2b/mouse-IgG2b,

rat-IgG2b/mouse-IgG3;

rat-IgG2b/human-IgG1,

rat-IgG2b/human-IgG2,

rat-IgG2b/human-IgG3[oriental allotype G3m(st) = binding to protein A],

rat-IgG2b/human-IgG4;

rat-IgG2b/rat-IgG2c;

mouse-IgG2a/human-IgG3[caucasian allotypes G3m(b+g) = no binding to protein A, in the following indicated as *]

mouse-IgG2a/mouse-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG3*-[CH2-CH3]

mouse-IgG2a/rat-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG3*-[CH2-CH3]

mouse-IgG2a/human-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG3*-[CH2-CH3]

mouse-[VH-CH1,VL-CL]-human-IgG1/rat-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG3*-[CH2-CH3]

mouse-[VH-CH1,VL-CL]-human-IgG4/rat-[VH-CH1,VL-CL]-human-IgG4-[hinge]-human-IgG4[N-terminal region of CH2]-human-IgG3*[C-terminal region of CH2: > aa position 251]-human-IgG3*[CH3]

rat-IgG2b/mouse-[VH-CH1,VL-CL]-human-IgG1-[hinge-CH2-CH3]

rat-IgG2b/mouse-[VH-CH1,VL-CL]-human-IgG2-[hinge-CH2-CH3]

rat-IgG2b/mouse-[VH-CH1,VL-CL]-human-IgG3-[hinge-CH2-CH3, oriental allotype]

rat-IgG2b/mouse-[VH-CH1,VL-CL]-human-IgG4-[hinge-CH2-CH3]

human-IgG1/human-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG3*-[CH2-CH3]

human-IgG1/rat-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG4[N-terminal region of CH2]-human-IgG3*[C-terminal region of CH2 : > aa position 251]-human-IgG3*[CH3]

human-IgG1/mouse-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG4[N-terminal region of CH2]-human-IgG3*[C-terminal region of CH2 : > aa position 251]-human-IgG3*[CH3]

human-IgG1/rat-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG2[N-terminal region of CH2]-human-IgG3*[C-terminal region of CH2 : > aa position 251]-human-IgG3*[CH3]

human-IgG1/mouse-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG2[N-terminal region of CH2]-human-IgG3*[C-terminal region of CH2 : > aa position 251]-human-IgG3*[CH3]

human-IgG1/rat-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG3*-[CH2-CH3]

human-IgG1/mouse-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG3*-[CH2-CH3]

human-IgG2/human-[VH-CH1,VL-CL]-human-IgG2-[hinge]-human-IgG3*-[CH2-CH3]

human-IgG4/human-[VH-CH1,VL-CL]-human-IgG4-[hinge]-human-IgG3*-[CH2-CH3]

human-IgG4/human-[VH-CH1,VL-CL]-human-IgG4-[hinge]-human-IgG4[N-terminal region of CH2]-human-IgG3*[C-terminal region of CH2 : > aa position 251]-human-IgG3*[CH3]

mouse-IgG2b/rat-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG3*-[CH2-CH3]

mouse-IgG2b/human-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG3*-[CH2-CH3]

mouse-IgG2b/mouse-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG3*-[CH2-CH3]

mouse-[VH-CH1,VL-CL]-human-IgG4/rat-[VH-CH1,VL-CL]-human-IgG4-[hinge]-human-IgG4-[CH2]-human-IgG3*-[CH3]

human-IgG1/rat-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG4-[CH2]-human-IgG3*-[CH3]

human-IgG1/mouse-[VH-CH1,VL-CL]-human-IgG1-[hinge]-human-IgG4-[CH2]-human-IgG3*-[CH3]

human-IgG4/human-[VH-CH1,VL-CL]-human-IgG4-[hinge]-human-IgG4-[CH2]-human-IgG3*-[CH3]

10. Method according to claim 1, characterized in that said bispecific antibody is selected from a heterologous rat/mouse bispecific antibody.

11. Method according to claim 1, characterized in that said trispecific antibody has a T cell binding arm, a tumour cell binding arm and a third specificity for binding to Fc receptor-positive cells.

12. Method according to claim 11,
characterized in that
said trispecific antibody is selected to be an anti-CD3 X anti-tumour-associated antigen antibody and/or anti-CD4 X anti-tumour-associated antigen antibody and/or anti-CD5 X anti-tumour-associated antigen antibody and/or anti-CD6 X anti-tumour-associated antigen antibody and/or anti-CD8 X anti-tumour-associated antigen antibody and/or anti-CD2 X anti-tumour-associated antigen antibody and/or anti-CD28 X anti-tumour-associated antigen antibody and/or anti-CD44 X anti-tumour-associated antigen antibody.

13. Method according to claim 1,
characterized in that
in said step c) after incubating the tumour cells with intact heterologous bispecific and/or trispecific antibodies the tumour cells charged with antibodies are prepared for reinfusion (short-term incubation).

14. Method according to claim 1,
characterized in that
in said step c) the incubation of the tumour cells with antibodies is performed together with mononucleated cells of the peripheral blood (PBMC = peripheral blood mononucleated cells), or mononucleated cells are added after incubation of the tumour cells with the antibodies

and the incubation is continued (long-term incubation).

A 15. Method according to claim 13 ~~or 14~~,
characterized in that
said tumour cells are incubated with the antibodies for a period of 10 minutes to 5 hours.

A 16. Method according to claim 13 ~~or 14~~,
characterized in that
said tumour cells are incubated with the antibodies for a period of 15 minutes to 120 minutes.

17. Method according to claim 14,
characterized in that
said mononucleated peripheral blood cells are incubated with the tumour cells and the antibodies for a period of 1 to 14 days.

18. Method according to claim 14,
characterized in that
said mononucleated peripheral blood cells are added in an amount of about 10^8 to 10^{10} cells.

19. Method according to claim 1,
characterized in that
said tumour cells are added in an amount of 10^7 to 10^9 cells.

20. Method according to claim 1,
characterized in that
said bispecific and/or trispecific antibodies are added in an amount of 2 to 100 μg.

21. Method according to claim 1,
characterized in that
said treating of the tumour cells in step b is performed by irradiation.

22. Method according to claim 1,
characterized in that,
said bispecific and/or trispecific antibodies are capable of activating the Fc receptor-positive cell whereby the expression of cytokins and/or co-stimulatory antigens is induced or increased.

23. Use of the tumour cell containing preparation according to claim 1 ~~or 14~~ in the prevention and treatment of tumourous diseases.

24. Use according to claim 23 for inducing an anti-tumour immunity.

25. Method according to claim 1 for the preparation of autologous tumour cells treated with heterologous bispecfic and/or trispecific antibodies for reinfusion
into the patient or the animals from whom the autologous

tumour cells have been obtained.

26. A pharmaceutical composition containing a tumour cell preparation obtained by the method of claim 1 ~~or 14~~.